The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 5 MARCH, 1939 NUMBER 2

BACTERIOLOGY IN THE SMALLER LABORATORY*

By J. L. LATTIMORE, M.D.

If one were to follow the literature for a year or two, then attempt to isolate the various bacteria, on the basis of the many recommended media, each laboratory would need to hire an extra technician, whose duties would be confined to making of these special media, in addition, extra refrigerators would have to be installed to carry an ample supply for immediate use.

Hailing from a state, renowned for its dust storms, goat gland specialists and drouths, a comparatively sparsely settled state and few cities, it means that such conditions alter the workings and procedures to be followed in the smaller laboratories. Due to these conditions it is not practical to have a highly trained bacteriologist in charge of the laboratory or as an assistant, for the technician often must do such odd jobs as assisting in the diet kitchen, give anesthetics, keep books for the office and various other side lines that do not occupy the usual leisure time of the technician in the larger laboratories. In Kansas there are some 50 laboratories located in towns of less than 8,000 inhabitants. In practically all instances, one of the local physicians is designated as "Director of laboratory," but seldom does he darken the door and too often dislikes to be around the laboratory for fear some other physician might ask him to do a bloodcount, about which he would not know how to proceed. It then evolves upon the technician, who I find, as

** Read before the American Society of Medical Technologists, San Francisco, Calif., June, 1939.*

a rule are well trained and capable, to develop all technic that shall be used. Over a period of some 20 years, contacting these laboratories through various means, I find a definite lack of uniform technic used, in facing many of the bacteriological problems.

I want no argument with the larger laboratories or institutions or those specially versed in the use of trick media but I present some ideas that may be of help to the workers in the smaller laboratory, that may direct them to the use of the minimum number of various media to be carried in stock. It most certainly is not practical to have to rush around and make up some media, after the patient or the specimen is in the laboratory.

So that there will not be too much waste, I would suggest that the media be put in screw cap test tubes. This prevents dehydration and by storing in the ice box, will remain suitable for use for several months.

A few points about blood cultures may be of value. First, may I impress upon you the importance of doing repeated cultures on a given case. I do not have available figures, but very often I find a positive culture on the second, third or fourth, after having a negative culture on the original. I suggest that a culture be made each day for six consecutive days, unless some other factor, such as marked anemia might alter the procedure. Draw 20cc of blood, put 10cc in a flask of brain broth, in a concentration of about 1:20 and the other 10cc is defibrinated and left in the flask and incubated. Numerous times a growth will be obtained in this defibrinated blood when the one with brain broth or blood agar is negative. Do not discard the cultures if negative for at least two weeks as several strains of streptococci grow rather slowly. Usually not much trouble will be encountered in identifying the organism, by transplanting to blood agar and the various sugars. Occasionally a rare bacteria will be obtained that will require extensive study with various differential media and animal inoculation. Just within the past 30 days, I repeatedly obtained a member of the diphtheroid group from a fatal case of septicemia, resulting from a traumatic injury to the head. Usually the smaller laboratory must depend upon a consultant to work these out.

In preparation of autogenous vaccines, care should be exercised in selecting the bacteria for the vaccine, as many of the contaminants produce rather powerful toxins and often this accounts for the very severe reactions in the patent. Personally I prefer solid media as it is easier to manipulate and does not

require repeated washings with salt solution to eliminate products of the media. I find blood agar and Loefflers media are satisfactory for practically all vaccines I am called upon to make. Plants are made upon two tubes of each media, one is cultured under reduced oxygen tension and the other under ordinary conditions. There are many methods of reducing oxygen tension, however I have found that an ordinary quart fruit jar serves the purpose. The tubes are placed in the jar and a small tallow candle is lighted, the flame will consume enough of the oxygen to give proper reduction, so when the flame goes out, satisfactory conditions are present. In the smaller laboratory most vaccines are made from the various skin lesions and many or most of the vaccines are made from staphylococci. I have found Bacto-crystal violet a very satisfactory differential media to use for pathogens. On this media, the pathogenic staphylococci will produce an orange or deep violet while the non pathogenic will produce a pale violet or more commonly, a white colony.

I have little to add to the literature on typing for pneumococci, except to stress the importance of this procedure as I know of nothing that is more dramatic than to have patient, gravely ill with pneumonia, to respond within a few hours to type specific serum. In making the slide preparation, be sure that the proportions of serum is great to the comparatively small amount of sputum. If it is desired to file the slide away for record, remove the cover slip after quellung is present, let the slide dry, then stain with methylene blue for two minutes. The quellung will remain present as a permanent record.

Although numerous bacteriologist have reported success with various media for the cultivation of the tubercle bacilli, in my own hands the results have not been so satisfactory. If no acid fast bacilli are found after repeated examinations and then with the antiformin concentration method, I find that the guinea-pig inoculation to be the most satisfactory method.

Usually direct examination of the spinal fluid will reveal the identity of the offending organism. Blood agar, under reduced oxygen tension is the most satisfactory media for growing the meningococcus. Five times during the past two years, I have obtained a rather odd, unidentified large Gram-positive, Gram-negative diplococcus from the spinal fluid. In each case the cell count was above 1000 per cu.mm and great numbers of bacteria were present. At times some would stain as Gram-positive and some as Gram-negative, maybe the next day they would all be Gram-positive, then the next day all would be Gram-

negative. In other words I was dealing with a diplococcus that was a border line staining type. I have had other bacteriologists work on this bacteria and none have been able to classify it. You are probably thinking that something is wrong with my Gram's stain. One of the best checks I know about for the Gram's stain is to carry on your laboratory shelf a vial of killed Colon bacilli for negative staining and a vial of Streptococcus for positive staining. Each time you have a Gram's stain, put a drop on each end of the slide and stain along with the unknown. First examine one and then the other and if both stain correctly, you can rest assured that the Gram's stain is working. Cases of meningococcus meningitis are reported with a cell count of less than 100 cells per cu.mm, however I have not had this experience. It is very important to do a differential cell count, when the cell count is increased. Clinically much importance is obtained from information as to the increase in the lymphocytes or polymorphonuclear leukocytes.

From a practical standpoint the smaller laboratory will find little satisfaction in trying to isolate the brucella group or the B. tularense. I suggest that you confine your efforts to the agglutination tests using blood.

There is no more perplexing problem in the laboratory than the proper classification of the Gram-negative extra-cellular diplococcus, with variations in size and shape. In the nose and throat and also the genitalia, there are seven Gram-negative diplococci that may be found (N. Sicca, N. perflava, N. flava, N. subflava, N. flavescens, N. catarrhalis and meningococcus in the nose and throat and the gonococcus around the genitalia). I will not go into colony appearance of these various non-pathogens. For meningococcus, the final diagnosis must rest upon the agglutination with a polyvalent anti-meningococcus serum. For gonococcus, the final diagnosis must rest with, obtaining a growth of Gram-negative diplococci, grown in the presence of 10% carbon dioxide at 37 degrees and then these bacteria producing acid in dextrose and not in other carbohydrates (maltose, saccharose and levulose).

In the urine, the usual problem is the differential diagnosis of the bacillus causing the infection. This is a very practical clinical problem as mandelic acid is used for B.coli infection and a mixed treatment if the infection is due to the aerobacter aerogenes. It is necessary then that a correct bacteriological diagnosis be made. Each of these bacteria will grow upon almost any laboratory media. At the end of 18 to 24 hours, stab

Figure No. 1—Diphtheroid character of the organisms. May remain in this form for several generations.

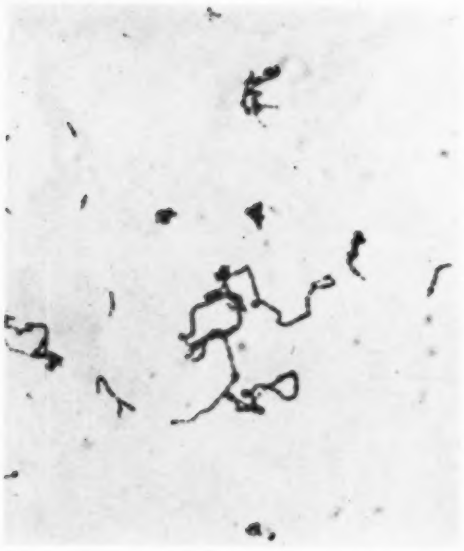


Figure No. 2—Shows both types of organisms. Diphtheroids predominate.

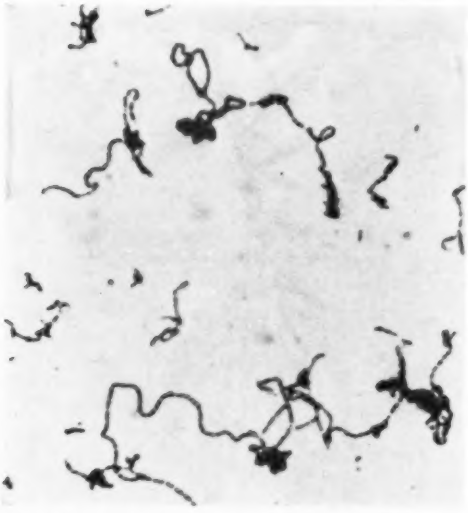


Figure No. 3—Diphtheroid forms still evident but definite chains of Cocci have formed.

transplants are made to saccharose agar. Escherichia coli will ferment the sugar while aerogenes will not.

Time does not permit me to go further and discuss many other problems of not so much importance.

In the smaller laboratory, I find that the following media, brain broth, agar, Loeffler's blood serum, eosin-methylene-blue, and four of the sugars furnish media for about 98% of the bacteriological work. Exercise special care and precautions in the handling of all specimens to prevent contamination and much diligence in identifying the strains of bacteria found.

A TECHNIC OF FLAGELLA STAINING

By MAURICE K. SCHIFFMAN, B.S., M.T.

U. S. Veterans Diagnostic Center, Fort Miley, California

Introduction

In all fields of science stained specimens are being preserved and filed for record, study, or comparison and prove to be a feature for exhibits. The technic of flagella staining has however given such disappointing results that many technicians have never used any of the many proposed methods. I have carried out several methods and modifications of flagella staining on Proteus vulgaris, Eberthella typhi, and Phytomonas campesterii in attempting to locate the steps in which one is most likely to have poor results, and develop a technic that may be used more routinely with a smaller percentage of failures.

Stain

The stain with which I had most satisfactory results is Gray's.

(1) Mordant:

Solution A

Potash Alum (sat. aqu. sol.)	5 cc.
Tannic acid (20% aqu. sol.)	2 cc.
Mercuric chloride (sat. aqu. sol.)	2 cc.

Solution B

Basic Fuchsin (sat. alc. sol.)	0.4 cc.

Solution A and B should be mixed less than twenty-four hours before using. The solutions will keep indefinitely when separate, but deteriorate rapidly after mixing.

(2) Stain: Carbol Fuchsin

Solution A

Basic Fuchsin (90% dye content)	0.3 g.
Ethyl alcohol (95%)	10 cc.

Solution B

Phenol	5 gms.
Distilled water	95 cc.

Mix solutions A and B.

Procedure

The general method of applying flagella stains requires first the procuring of young and vigorous cultures. In general, organisms are best suited for staining when grown on surface slants of standard agar. If old cultures are used they should be transferred daily until their vigor has been restored. Motility may easily be determined by examining a hanging drop slide. Organisms from old cultures are sluggish in movement while the motility of vigorous young organisms is very pronounced.

Although varying periods of incubation time may be allowed before removing the organisms from the culture tube to the slide, I have obtained my best results by using a loopful of the condensation water from a twenty-four hour culture at 37°C which disregards the optimum temperature for the organism, believed by some to be essential in obtaining good flagella stains. With Protozoa isolated from a hay infusion, it was not necessary to take the customary time precautions as the flagella remained on the organisms under practically all conditions.

A very important part of the procedure is to have the cover glasses and slides clean. By cleaning is not meant to wash and dry with a towel, but to boil in cleaning fluid and then rinse in weak hydrochloric acid followed by distilled water. This method will remove all traces of dirt. The slides and cover glasses should then be placed in alcohol until ready for use as it is an easy matter to dry the cover glass over the flame while holding it in a forceps. The slides may be dried by baking on wire gauze over a bunsen burner just before using.

A. Preparing the Organisms

The warm cover slip is now ready to receive the organisms, which should be suspended in distilled water at 37°C and added to the glass in tiny drops or streaks. Most satisfactory for me was the technic of gently expelling the suspended organisms on a warm cover slip from a very fine capillary tube. Any cover glass showing signs of grease should be discarded as the organisms will adhere to a single spot and be clumped too thick for satisfactory staining and examination. The drops or streaks should be so thin that they will dry almost instantly with as small an amount of heat as possible; preferably from the cover glass itself. When fixation by the use of a flame is employed it will be found, nine out of ten times, that the delicate and sensitive flagella have been destroyed.

B. Applying the Mordant

After the film has dried the mordant should be applied from

five to ten minutes. I found that five minutes was sufficient for any of the three organisms. Upon examining some of the cover slips after staining, those on which mordant was allowed to remain as long as ten minutes showed no flagella or detached flagella. There is nothing to prove this trouble is due to the time factor, as the difficulty in locating the flagella after mordanting and before the stain is added makes it almost impossible to be sure of their original presence and condition.

C. Applying the Stain

The stain is added to the cover slip after removing the mordant and allowed to remain approximately five minutes before being gently rinsed in distilled water. (I usually remove the mordant by flushing with a few drops of stain). Air dry and mount in balsam.

I could find no difference in the results by staining cold or slightly heating, but as the flagella are sensitive to heat I believe it best to stain cold and thereby eliminate any chances of overheating. However, if one chooses to use some other type of stain he may find it essential to use heat, and if so will get best results by warming the stain rather than the slide or cover slip.

Conclusion

(1) The difficulties confronting one in applying flagella stains are not in the ability of the technician to use the stain, but for the most part in the age of the culture and in the cleanliness of the cover glasses and slides.

(2) A simple satisfactory routine technic can not be definitely outlined due to the sensitiveness of the flagella and the little we know of their relationship to the internal cell structure.

(3) I believe any technician can obtain satisfactory results with Gray's technic and modified application as outlined in the above procedure.

BIBLIOGRAPHY

Gray, P. H., 1926: A Method of Staining Bacterial Flagella. Journal of Bacteriology, 12, 273-4.

Manual of Methods for Pure Culture Study of Bacteria. 1934, Leaflet 4, Staining Procedures, p. 16.

Burdon, 1932: A textbook of Bacteriology, p. 517.

SIMULTANEOUS CUTTING OF SEVERAL SECTIONS IN THE PREPARATION OF HISTOLOGICAL SLIDES

By SISTER M. SIXTA, M.T.

St. Elizabeth Hospital, Lincoln, Nebraska

The preparation of slides becomes tiresome and time consuming if one has 12 to 22 sections from one autopsy or as many as 6 or 10 from one operation. Cutting these sections singly with the microtome takes considerable time and it is often very difficult to get even 6 separate sections on one slide. Using several slides, for each individual case, when all histological preparations are kept indefinitely wastes space in the laboratory slide file.

We have found by experience that here one can save considerable time by using a new embedding material, Fisher tissuemat. The one we use routinely has a M.P. 54-56°.

We leave our sections in the first bath of tissuemat overnight. Then they are placed for one hour in a second bath of tissuemat, after which they are embedded in tissuemat in paper boxes. We place as many as 6 sections of different organs in the same box, and the microtome will cut perfect ribbons. It takes less time to cut this one block, than 2 or 3 single sections. It is quite simple to get these ribbons on a slide, and one can easily place as many as 14 small sections on one slide, and still have one inch free to write down name and numbers. It will happen, once in a great while, that one of these sections will give difficulty in cutting, but not more than single sections do. Every technician knows the trouble a flat section will occasionally give, by falling out of the embedding material. With tissuemat I found it was always enough to dig a little groove in the old block with a needle and press the section into it. Let it lie on ice a few minutes, and it will cut perfectly.

We keep the tissuemat constantly melted in the paraffin oven. When remelting used tissuemat, we found, that one has to be careful to pick out all pieces of tissue. Small parts of tissue, left in the melting beaker, spoils the preparation.

This method has saved us very much time, and I think that other technologists might like to know about it, too.

CULTURE REPORTS ON BILE AND BILIARY DRAINAGE: A COMPARATIVE ANALYSIS*

By ANNETTE M. CALLAN, M.T.

316 Carver Hall, Frankford, Phila., Pa.

Stimulus for this study, entirely personal interest at the time begun,** was the marked variance in growths and types of organisms recovered on bile specimens, received by the laboratory from operating room, following cholecystectomy, and those recovered from biliary drainage specimens, from gastric clinic service, on "study" cases. Several months of close observation of such results, gave rise to considerable doubt as to the value of reports on one service or the other.

It was felt that, insofar as patients' histories were concerned, such records were probably of little value to the clinician; were probably not indicative of patients' conditions. If embodied in literature, they might be grossly misleading.

Technologists today may have results not unlike those at the time this study was begun. Pressure of a large amount of work requires that material be accepted as submitted, be hurriedly studied and reported upon. There is usually little opportunity to analyze one's own work, as a "series of cases" and subject it to critical analysis by true and false tests.

This study is a report on work previously charted. It is not one of pre-arranged, lined-up cases, with planned study, almost ideal conditions and animal study, the usual procedure in a research problem.

It is an analysis of culture studies, routinely reported to clinicians, under conditions existing and accepted at the time specimens came to the laboratory.

The plan was to study factors *outside* the laboratory and *within* it, likely to re-act favorably or unfavorably on the specimens and influence the final report. Some reports were unquestionably false, others were to be accredited.

---

* *Read before the American Society of Medical Technologists, San Francisco, Calif., June, 1938.*

** 1933-34; Dr. Frank W. Konzelmann, Pathologist.

Attempts are here made by comparison, approximation and deduction, to evaluate the reports.

Factors Outside the Laboratory

Factors outside the laboratory, recognized and checked, were roughly three:

(1) training and number of persons collecting specimens
(2) method of collection and container used
(3) possible failure of growth, due to
    (a) delay in delivery of specimen for study
    (b) exposure of specimen to cold for long periods
    (c) inhibitory (?) influence of bile itself.

Factors Inside the Laboratory

Factors inside the laboratory, recognized and checked, were roughly four:

(1) experience and technic of technologist culturing specimens
(2) technic employed in culturing specimens
(3) sterilization of supplies
(4) appropriateness of media.

Specimens of bile from operating room were collected by the surgeon and/or his assistants. They were aspirated by syringe into a sterile, wide mouth test tube (about 50 c.c. capacity), stoppered by a cotton plug and so sent to the laboratory. Amounts ranged from 2 c.c. to as much as 40 c.c., the average being about 10 c.c. Material was designated as "bile," and was invariably clear, dark green, viscid fluid. Diagnoses were roughly "cholecystitis," "cholecystitis with cholelithiasis," and "gall-bladder disease."

Often specimens were delayed before receipt by the laboratory, due to pressure of work. Such delay ranged from four or five hours, to as long as three days, when placed in an ice-box and overlooked.

Specimens of "B. bile" by biliary drainage, from clinic, were collected by three groups: an experienced technologist in charge of patients in private rooms in hospital, at their homes or reporting to physicians' offices. Two technologists, trained especially in this type of work, were in charge of bed patients in hospital wards and clinic ambulatory patients. Two students, in training for two months in this service, were acquiring experience.

Results by culture seemed in proper relation to experience of these workers.

Specimens were drained directly into "fool-proof" flasks

(Lyon's type). The amount was 20 drops into 150 c.c. of fluid medium (0.2% dextrose hormone broth). Such specimens were delivered promptly, after collection, and were incubated overnight, unopened.

Within the laboratory, all specimens were promptly cared for. One technologist handled all specimens throughout the study. All were handled with care and by approved technic. Sterility of flasks issued to the clinic, and media used for culture study, was established.

Technic of Culture

Bile received from operating room was centrifuged for thirty minutes, at high speed, in the tube submitted. All but 1 c.c. of bile was poured off into another sterile test tube and retained. No specimen showed gross sediment. Cultures were made from the assumed sediment: upon plain and blood agar plates (by streak) and 6 loopfuls were inoculated into 10 c.c. of hormone broth and into 10 c.c. of Rosenow's brain broth. After preparing spreads for Gram stain, 10 c.c. of hormone broth were poured over all of the remaining sediment.

Incubation was overnight (17-23 hours). Gross inspection followed in the morning and necessary detailed studies were carried on to completion of the case.

Results of Above Procedure

Clinic specimens, representing but a *small* amount of *uncentrifuged* bile, in a *large* fluid volume, yielded a high incidence of luxuriant growths, usually of mixed types, and a high percentage of apparent gross contamination by a large Gram positive spore, of B. subtilis type.

Operating room specimens, *centrifuged*, and despite *manipulation* of collection and culture, showed a high percentage of negative cultures, a low incidence of spore contaminations and positive cultures were usually of a single type. No specimen showed organisms present by Gram stain.

Histologic study of gallbladders in this series showed very few instances of pathology.

It is to be emphasized that specimens here considered, represented bile from the gallbladder and bile from the duodenum. Allowance must be made for organisms recovered along the tract in drainage cases. Many of these organisms are rapid growers, acid formers and likely to retard or completely inhibit growth of more delicate organisms of pathogenic type. This aspect is an important one to consider if vaccine be requested on such material. A twenty-four hour growth of mixed organisms, in fluid

medium, very often results in loss of the important organisms when subcultured to a plate.

Accession records of the laboratory showed 102 specimens of bile had been studied during the calendar year chosen for this analysis*. An even 100 cases were selected for consideration. These were derived as follows:

Operating Room service, 30; Gastric Clinic service, 70 (ambulatory, 45; hospital bed patients, 14; outside private practice, 11).

*Organisms Recovered***

Organisms recovered were:

Total cases	Operating room 30	Clinic 70
Negative cultures	23 (76.6%)	7 (10%)
B. subtilis	2 (pure)	14 (pure) 6 (in combination)
Non-haemolytic streptococcus	2	4
B. proteus	0	1
B. coli communis	2	20
B. coli communior	0	11
B. lactis aerogenes	0	1
Saccharomyces cerevisiae	0	1
Staphylococci:		
Haemolytic aureus	0	3
Non-haemolytic aureus	0	3
Sl. haemolytic albus	1	4
Diphtheroids	1	3
M. catarrhalis	0	1
B. mucosus capsulatus	0	1
B. typhii	0	0

This table shows 15 types of organisms. Disregarding the B. subtilis, operating room specimens showed 5 positive, with an incidence of 7 organisms,—non-haemolytic streptococcus and B. coli occurring once, and B. coli and a diphtheroid occurring once.

* 1933.

** Comparable to those listed in text, Kolmer and Boerner—Approved Laboratory Technic, 1st ed., 1931; 2nd ed., 1938. D. Appleton-Century Co.

Considering the drainage cases,* the 15 types showed an incidence of 68 growths, usually in combination of two or three, on 49 specimens.

It is of interest to note some combination of mixed growths from the drainage specimens:

B. subtilis with B. coli communis	thrice
B. subtilis with non-haemolytic staphylococcus albus	twice
B. subtilis with non-haemolytic streptococcus	once
B. coli communior with non-haemolytic staphyloccus aureus	thrice
B. coli communior with saccharomyces	once
B. coli communis with non-haemolytic streptococcus and diphtheroid	once

Each combination shows at least one organism of no significance or of questionable origin. Note survival of some types, after subculture from flask to plate, despite other organisms present.

It is not to be implied or inferred, that because so few cultures were positive in the surgical group, that operation was not indicated or not found to be warranted. Many factors, apart from bacteriological findings, must necessarily add weight for or against surgery.

Name analysis failed to show even one instance of pre-operative and surgical specimens submitted on the same patient within the year span analyzed. Search of records, three months prior to and subsequent to this year, showed one operation had followed a December drainage. Results by culture were comparable to those listed: a mixed growth in drainage, surgical specimen negative.

Based on the foregoing information, a study was planned to determine the following:

effect of delay (at room temperature)
effect of delay (with exposure to cold)
inhibitory effect of varying concentrations of bile

The object was to ascertain whether some growths were being *lost* in culture, also to learn whether organisms, of a type likely to be present, were recoverable when affected by these influences.

Study to Approximate Conditions

The following organisms were selected for this check study. Isolated strains from bile cultures were: non-haemolytic strep-

* High incidence of B. subtilis was traced to inadequate sterilization of drainage tube. shown by culture of inside washings of the tube. Change in sterilization method materially reduced presence of this organism in cultures.

tococcus, B. coli communis and non-haemolytic staphylococcus (aurantiacus type). From stock laboratory cultures, the following were used: B. typhii (Rawlings'), Para B, (Cool's), Br. melitensis (var. abortus, #486, USPH.)

All were carried on slants of appropriate media until completion of the study. When needed for inoculation of the "dummy" bile specimens, 24-hour broth cultures were used. Br. abortus was emulsified in saline, from the solid medium slant. All were diluted to approximately 200,000 organisms per c.c. (by nephelometer). Four loopfuls were used for seeding the bile.

Pooled human bile from operating room specimens, previously showing no growths, had been retained for use as these "dummy" specimens. Sterility was proved.

Possible Effect of Delay (room temperature)

1 (a) Each of the foregoing organisms was seeded into 10 c.c. of human bile and let stand eight hours at room temperature. Then regarded as human specimens, just delivered to the laboratory, each was centrifuged and carried through routine culture procedures upon plates and into fluid media. There was no evidence of organism by Gram stain from sediments.

Overnight incubation showed characteristic growths by gross inspection and by stain. *Br. abortus was not recovered.*

(b) A duplicate set-up of the foregoing, let stand at room temperature for eight hours, was then placed in the ice-box for forty-eight hours. Then regarded as human specimens, just received, all organisms were carried through routine culture procedures. All were recovered with characteristic growths, *except non-haemolytic staphylococcus aureus,* which appeared *haemolytic.* Two organisms failed to grow: non-haemolytic streptococcus and Br. abortus.

Checked a second and third time, results were comparable.

Possible Inhibitory Effect of Bile

Organisms used above were inoculated into 10 c.c. of human bile and let stand eight hours.

For this test, bile enriched media were prepared, as follows: 1 c.c. of bile added to 9 c.c. of hormone broth; 0.5 c.c. of bile added to 9.5 c.c. of Rosenow's brain broth; 0.1 c.c. of bile added to 9.9 c.c. of Hiss water serum; 10 c.c. of pure bile. These four tubes were regarded as one set-up. Each organism previously seeded into pure bile, was then inoculated into the set-up of four tubes. Incubated overnight and streaked to plates the following morning.

Growths were characteristic. Again, haemolytic staphylococcus was recovered instead of non-haemolytic staphyloccus. Neither the Br. abortus nor non-haemolytic streptococcus was recovered.

Checked a second and third time, results were comparable.

There was a difference in growth characteristics in fluid media. Organisms grown in concentrated and bile enriched media tended to grow at the base of the tube. This was not evident in fluid media without bile enrichment.

Whether due to slight change in pH of medium, to surface tension or preference for lessened oxygen, this aspect of growth in fluid could not be determined.

Deductions

Due to special requirements of pH and special medium enrichment, Br. abortus is probably not recoverable by routine culture procedure.

Recovery of streptococcus after slight delay, at room temperature and its loss in culture when delayed and exposed to cold, is a point for serious consideration in negative reports.

Recovery of other organisms, despite these influences, may be expected in routine procedure.

Culture changes and haemolytic properties of organism, grown in bile enriched medium may effect the character of report on the culture.

Literature

Although there is a vast amount of literature on gallbladder conditions, chiefly aspects of surgery, there is relatively little on its bacteriology and much of that is foreign.

The present method and apparatus for collection of biliary drainage is generally accepted. It is doubtless limited in value for bacteriology. Performed with due care, Kolmer & Boerner regard specimens as usually acceptable for bacteriological examination. As opposed, D. M. Lyon's opinion is that it is not very reliable.

Organisms recovered, as in this study, are within the range of those listed in standard text (Kolmer & Boerner).

Wide diversity of opinion as to the nature of gallbladder infections is emphasized by Wilkie.

Material best suited for culture study is shown by Rosenow and also by Thorsness, to be cultures from submucosa and cystic node, rather than bile alone from gallbladder. Thorsness holds

that culture of the cystic node is simpler than from submucosa.*

Failure of growth from pathological gallbladders, is reported by Wilkie to be due to some factor in technic used in the culture. He also reports inhibitory effect of even a few drops of bile, when added to a culture.

Culture variances are reported by Wilkie, citing differences in sugar reactions of streptococcus recovered from bile and that recovered from submucosa. Thorsness reports a greater virulence in streptococcus recovered from the wall than from bile. He also reports growth variances, following delay: scant growth, which when subcultured showed abundant growths which settled, yielding short-chain, diploid, non-haemolytic streptococci, smooth type.

Organisms of major interest. The first three listed on Table 1, are apparently the only ones of major interest. Rosenow holds that other organisms (producing no lesions in his studies) are to be regarded as accidental invaders.

Streptococci. Rosenow demonstrates recovery of streptococci with greater frequency from the wall than from the bile. His conclusion is that organisms reach the gallbladder by way of the arterial blood. Wilkie reports bile usually negative; wall usually negative; streptococci recovered in 42% of cases from submucosa and 80% of cases from the gland.

B. pyocyaneus. Thorsness reports this as an infective agent in connection with some instances of stone. Reports it found four times more often in the wall than in the bile and from two to four times more often than in the macerated stone.

B. coli. Hurst, Knott & Venables (cited by Wilkie) reports this organism as the one most frequently isolated from duodenal bile, as opposed to view held by Rosenow. Wilkie reports recovery of this organism in 6% of cases. It was recovered from submucosa when not recovered from bile. Found three times associated with multiple stone.

Clostridium welchii.** Likely recovery of this organism is stressed by Thorsness. Gordon-Taylor & Whitby report it nine times in 50 cases. Rosenow reports it six times in 48 cases.

* Submucosa:—meaning intended to signify subepithelial layer. There is no true submucosa in gallbladder comparable to that found in stomach and intestine (Halpert).

** Method for recovery. Macerate strips of submucosa and mucosa. Use anaerobic blood plate to determine type. Use glucose broth; to identify, use Blair & Wilson's medium to elicit iron sulphide reaction; use sterile deep milk to elicit stormy fermentation in 24 hours. Thorsness reports organism of low virulence.

Summary

1. Drainage by one experienced technologist was found most satisfactory for culture work as it eliminated possibility of gross contamination.

2. Specimens handled by surgical group and laboratory technologist showed a low percentage of contaminations.

3. Several types of media used yielded satisfying results; growths were constant and in about like proportion.

4. Minimum direct drainage of bile into flasks yielded a greater number of growths, usually mixed, and a greater number of contaminations.

5. Bile specimens from gallbladder, yielded few growths, usually of a single type.

On Approximated Studies

6. Common pathogens were recovered under aerobic conditions, in presence of bile.

7. Inhibition, not evident except against streptococcus in bile enriched media.

8. Delay and exposure to cold. Streptococcus and Br. abortus were not recovered.

9. Preference for lessened oxygen. More marked in concentrated and bile enriched media than in non-enriched bile fluids of same kind.

Conclusion

Present method and apparatus for drainage must continue until better are available.

Bacteriologic studies on bile from gallbladder and drainage, are not a satisfactory index to gallbladder infection.

Infecting organism is best proved only after surgery.

BIBLIOGRAPHY

Amanica, F.: Bacteriologic observations on human bile, with special reference to streptococci. Policlinico (see Prat.) 41: 243-247, Feb. 19, '34.

Amory, O. T.: Report on 100 consecutive cases. Virginia M. Monthly, 61: 296-299, Aug., '34.

Castro, Villaneuva W.: Findings in so called Bacterium typhi flavum in bile of cattle. Zentalb. f. Bakt. (Abt. I.) 132: 1-4, July 2, '34.

Gordon-Taylor, G. & Whitby, L. E. H.: A bacteriological study of fifty cases of cholecystectomy with special reference to anaerobic infections. Brit. J. Surg., 1930, XVIII, 78-83.

Halpert, B.: Morphological studies on gallbladders. Bull. Johns Hop. Hosp., 1927, XL, 390.

Hernández, A. Curbelo, Insua, Cartaya, & Gomez, V. Toyoo: Bacteriology of cholecystitis; studied in 45 exterpated gallbladders. Rev. de med.

y. cir. de la Habana. 39: 461-485, July 31, '34 (comment on Wilkie's article).

Holbrook, A. A.: Non-typhoid cholecystitis in childhood, with report of unusual case of cholangelitis. Am. J. dis. Child. 47: 836-849, April, '34.

Illingsworth, C. F. W.: Types of gallbladder infection. Brit. J. Surg., 1927, XV, 22.

Kolmer, J. A. & Boerner, F.: Approved laboratory technic. 1st ed., New York, D. Appleton-Century Company, 1931; 2nd ed., 1938.

Lyon, D. M.: B. coli infection: etiology and treatment. Brit. Med. J., 2, 458, Sept. 8, '34.

Mortgau, H. G., Duran. C. & Tapla, J.: Clinical value of bacteriologic study in detection of biliary allergy. Arch. espan. de enform. y de la digest. y de la nutricion. 17: 149-155, March, '34.

Rosenow, Edward C.: Bacteriology of cholecystitis and its production by injection of streptococcus. Jour. Amer. Med. Assoc., 903: Sept 12, '14.

..... Collected papers, Mayo clinic, 1919, 819.

..... Support in evaluation. Causation of gastric and duodenal ulcer by streptococci. Mayo Found., Rochester, Minn., pub. Mar. 28, '14.

..... The etiology of cholecystitis and gallstone and their production by intravenous injection of bacteria. J. Infect. Dis., 1916, XIX, 527-556.

Thorsness, E. T.: Cholecystitis, virulence and spore formation of Cl. welchii. Surg., Gyne. & Obst. 59: 752-755, Nov., '34.

Wilkie, A. L.: The bacteriology of cholecystitis: A clinical and experimental study. Brit. J. Surg., 1928, XV, 450-465.

A NEW AID IN TITRATION

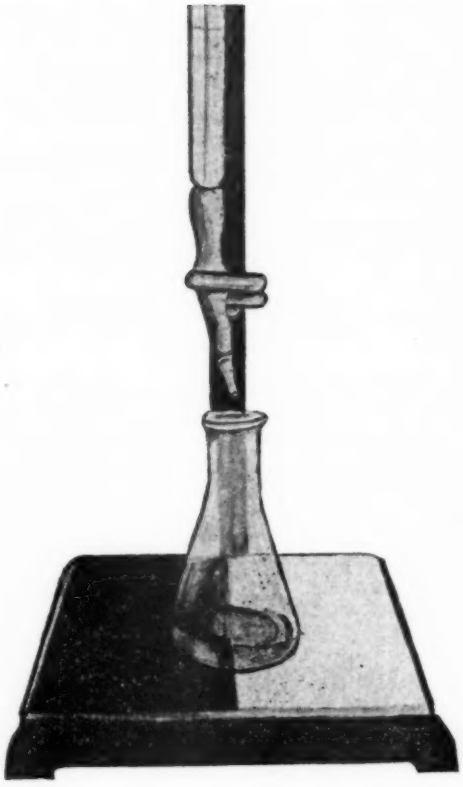
By GEORGE R. SLATER

Laboratory Technician, Robinson Clinic, Kansas City, Missouri

Just recently I conceived a new idea in titration which I believe should prove of interest to everyone connected with Laboratories, an idea which is very simple, yet, one that is of great help. In titrations previously for certain types of work it has been necessary to place a sheet of white paper on your stand to be able to differentiate your sharp points. So, I therefore painted one-half of my stand white and left the other side the conventional black color.

In the past two months I have found this new method very satisfactory and of great help when one is quite busy. With this idea work is simplified greatly, and one is able to do better work, faster than before.

Illustration of stand as painted is reproduced on following page.



EDITORIAL

"ST. LOUIS AND OL' MAN RIVER"

Convention City for A. S. M. T. Session, May 18-20, 1939

St. Louis a miracle city—the new St. Louis, a rich, modern metropolis, the capitol of commerce and industry in the Middle West, but rather yet in glorious tradition and romantic evidence of past generations.

It was on the evening of February 14, 1764, that a little band of French pioneers first landed on the west bank of the Mississippi River at what is now the foot of Walnut Street in St. Louis. For many days, patiently fighting the current, they had poled and dragged their heavy craft up the great river from Fort de Chartres, sixty miles below. Wearied by their labors, they slept that night on their boat.

Like the landing of the Pilgrim Fathers, the coming of this "First Thirty," as they became known in colonial days, provided a mile-stone which marked the beginning of an empire. For when, on the following morning, August Chouteau led his men across the sandy beach and up the plateau overlooking the river, pointing out to them there a line of blazed trees, the ringing blows of axes soon sounded through the woods, and the building of St. Louis began. Then and there was born the spirit of a community.

The previous year a far-sighted engineer named Laclede had conceived the idea of a permanent settlement in some favorable river location. Searching for the ideal spot, he, accompanied by August Chouteau, explored the Mississippi North and South. And, as the still preserved record relates, "He fixed upon this place, marked with his own hands some trees, and said to Chouteau, 'You will come here as soon as navigation opens, and form a settlement after the plan which I shall give you. For here may well develop one of the finest cities in America, since here are such unusual advantages of location and of central geographical position.' "

Those were indeed pioneer days, days when the European powers, England, France and Spain contended for a continent. At that time neither cities nor towns existed in all the silent wilderness of the Mississippi Valley. Here and there, hundreds of miles apart, roughly stockaded and scantily garrisoned forts constituted the only outposts of civilization, the sole refuge against Indian attacks.

Frontier Lines there were none. Life in the New World was a continual struggle for existence.

Other expeditions, French and Spanish, soon sought to overshadow the little settlement of St. Louis. A Spanish Fort was built a short distance to the north. Yet so well had Laclede chosen, and so energetically had his followers labored, that these competitive efforts gradually merged with St. Louis itself. Within three years its colonists, by sheer force of spirit, had established valuable fur-trading monopolies with the twenty-eight principal Indian nations, including not only those west of the Mississippi, but also east of the river and even as far north as the Great Lakes. These the Englisn tried in vain for many years to break.

Within five years the fur trade of St. Louis had grown to the amount of $80,000 annually, a great sum in those days. That trade was the commercial cornerstone, the basis of prosperity. Every year thereafter saw the city's radius of influence lengthen. Up the Mississippi and Missouri crept a line of outposts. St. Louis became the gateway of the stream of migration, the starting point of expeditions in all directions. Some of these were military, establishing forts; some scientific, to explore and exploit; more were to establish communities, to open commercial avenues. The Lewis and Clark Expedition in 1804, opening the Northwest, was one of these. So, too, the Frenchmen of St. Louis paved the way for the American occupation of Louisiana. A branch of the Chouteaus started Kansas City,, Robidioux, of St. Louis, established St. Joseph. One of the Menards founded Galveston. A hundred Western Cities and towns owe their beginning to St. Louisians..

With the "Louisiana Purchase" in 1803, all that vast stretch of territory which is now the central and western part of this country came into national possession, more than doubling the area of the United States. Meanwhile, St. Louis has steadily grown. Seven years after its incorporation as a city in 1823, its population was 4,977, ranking 44th among American cities. In 1833 it was in 20th position and growing fast.

Missouri became a state in 1821, and, in time, became the central state of all the Union. Two states away, to the south, today, lies the Gulf of Mexico. Two states north is the Canadian Line. Five states east is the Atlantic. Five states west, the Pacific. Thus, Missouri, and St. Louis, its chief city, is the geographical heart of the Union, they very center of its life and activities.

1811 marked the appearance of the Mississippi Steamboat. Five years later the first steamboat came up the river to St. Louis. For half a century thereafter the river trade grew by leaps and bounds. Just prior to the Civil War this river traffic was at its height. Hun-

dreds of the old-time steamers, their decks piled high with cotton, daily ploughed the Mississippi. The steady, chugging beat of their paddles and the hoarse boom of their giant whistles awoke the echoes throughout the valley. Millions of dollars were invested in the river fleet. St. Louis was at that time the leading city in the West.

On the borderland between North and South, Missouri suffered cruelly from the Civil War, more than one-tenth its battles being fought upon Missouri soil. The great current of traffic, which up to that time flowed north and south, was abruptly broken. The tides of trade turned east and west, served by rails instead of rivers. During the reconstruction period St. Louis temporarily lagged, yet it soon caught the cadence of the shriller whistles and moved on, losing but one rank in the procession of American cities. And today, the sixth largest manufacturing city, St. Louis, with its seventeen trunk lines operating twenty-five lines of railroad, has become America's second greatest railroad center, with a reborn river traffic greater than ever dreamed possible, and with developing possibilities which only the most farsighted can conceive .

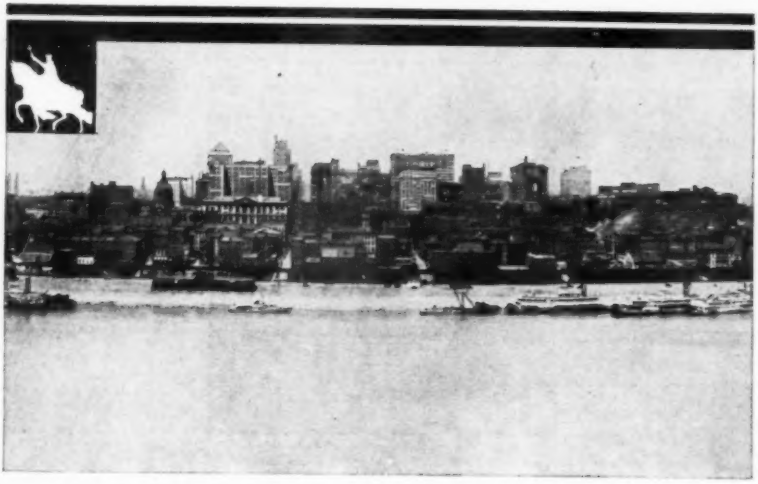
St. Louis has many advantages not afforded other cities.

Situated as it is, it is the most easily accessible city in the United States. The Terminal Railroad Association of St. Louis has the largest unified freight and passenger terminals in the world. It owns and operates the great St. Louis Union Station by which all passenger trains enter and leave the city. It has more than 400 miles of track, handles 4,660,000 freight cars and 650,000 passenger cars annually, serving 1,500 industries direct from its own tracks. It operates 6 belt lines, 175 switching engines, and has interchange connections with 27 railroads at more than 50 different points, thus utilizing the combined car supply of all these sources and insuring St. Louis shippers a maximum of shipping facilities at all times. You can move your goods in and out of St. Louis in best of fashion.

Because the Mississippi River has played such a significant part in the entire history of our city, it is perhaps one of the first things that the visitor wishes to see. With its tugs and steamboats, its barges and tows, its pleasure crafts, its ferry boats and mighty bridges, all flanked by the elevated railroad and the skyscrapers of the nearby business district, it presents an unforgettable picture. The old section of St. Louis adjacent to the river, still interesting, forms the district referred to by Charles Dickens in his "American Notes" as the French Quarter.

St. Louis is at the center of the Mississippi River inland waterways system, the largest inland waterways in the world. This river system consists of a series of navigable rivers and canals having a

Old Court House, St. Louis



Skyline View of St. Louis from River Front

Olive Street Canyon
St. Louis

total of 13,394.42 miles, which is more than any single railroad system in this country. More than 9,000 miles of this system may be considered main trunk-line waterways. These trunk lines may be classified into waterways of 9 foot channels or deeper and those of 6 foot channels or deeper.

The Mississippi River system connects by water 29 of the principal industrial cities of 20 states in the Mississippi Valley, with a total population of 11,000,000 and affects by joint river and rail rates a population of more than 50,000,000. The proposed increase in depth of channels to Minneapolis, St. Paul and Kansas City, will give a unified system of 9 foot waterways from the Gulf of Mexico to the Great Lakes and from Kansas City to Pittsburgh, with St. Louis as the geographical and control center.

Long-haul freight service on the Mississippi River is afforded by the Inland Waterways Corporation operating the Mississippi-Warrior Service, a Federal barge line south to New Orleans and north to Minneapolis and St. Paul. The service south is available throughout the year; north, eight months of the year.

Short-haul business is handled by the packet line. Several companies handle this end. Rates via the Federal barge line include the absorption of switching charges from and to industries in the local switching district. All-water rates are unformly 20% less than all-rail rates, except the rates on grain show a larger saving than 20%. The barge line issues the standard form bill of lading, which affords the same protection to water shipments as is enjoyed by shipments moving all-rail.

St. Louis, the most accessible convention city in America, should attract a majority of the A. S. M. T. members who are promised a program of special interest.

BOOK REVIEW

THE MEDICAL APPLICATIONS OF THE SHORT WAVE CURRENT by William Bierman, M.D., Attending Physical Therapist, Mount Sinai Hospital, New York City; Assistant Clinical Professor of Therapeutics, New York University College of Medicine. Including a Discussion of its Physical and Technical Aspects by Myron M. Schwarzschild, M.A., Physicist, Beth Israel Hospital, New York City; Instructor of Physics in Radiology, New York University College of Medicine. 379 pages with 25 plates and 60 figures. William Wood & Company, 1938, Baltimore, Md. Cloth, $5.00.

This is an authoritative work on the clinical application of the short wave current or what has come to be known as diathermy. The author is of the opinion that its beneficial effects are due solely to the physiological responses resulting from heat production although he holds an open mind regarding the contentions of others that the important result is an athermal, specific one due to the mechanical or electrical nature of the high frequency oscillations. A third group maintains that the results are due to a combination of the two.

A lengthy discussion of the physical and technical considerations of the short wave current is given by Myron M. Schwarzschild, M.A., a physicist thoroughly familiar with the medical requirements of short wave diathermy.

The author discusses the physiologic responses to local heat and local short wave currents of the various tissues of the body such as the blood, blood vessels, lymph system and tissues, digestive and nervous systems, etc. He uses freely the opinions, experiences and results of experimentation of others who are outstanding in this field. Controversial topics are presented fairly and without bias. Following a discussion of the technique of using the short wave current the clinical applications are given. The author's own experiences and results as well as those of many others are used for a consideration of the clinical application of short wave therapy in more than a hundred diseases. The author states that diathermy is not to be used to the exclusion of other recognized methods of treatment but is employed in conjunction with other accepted procedures. In some conditions, however, this method of therapy is adequate in itself. Every practitioner using short wave therapy will find this book most helpful.

NEWS AND ANNOUNCEMENTS

"The American Physicians' Art Association composed of members in the United States, Canada, and Hawaii, will hold its second Art Exhibit in the City Art Museum of St. Louis, May 14-20, 1939, during the annual session of the American Medical Association. Art pieces will be accepted for this art show in the following classifications: (1) oils both (a) portrait and (b) landscape; (2) water colors; (3) sculpture; (4) photographic art; (5) etchings; (6) ceramics; (7) pastels; (8) charcoal drawings; (9) book-binding; (10) wood carving; (11) metal work (jewelry). Practically all pieces sent in will be accepted. There will be over 60 valuable prize awards. For details of membership in this Association and rules of the Exhibit, kindly write to Max Thorek, M.D., Sec'y., 850 Irving Park Blvd., Chicago, Ill., or F. H. Redewill, M.D., Pres., 521-536 Flood Bldg., San Francisco, Calif."

The 68th Annual Meeting of the American Public Health Association will be held in Pittsburgh, Pa., October 17-20, 1939, with headquarters at the William Penn Hotel.

Dr. Reginald M. Atwater, Executive Secretary, in announcing the dates, calls attention to the important issues facing the public health profession and predicts a year of great expansion in the responsibilities of health officers and health workers generally.

Dr. Atwater says: "The Annual Meeting of the American Public Health Association grows larger, more important and more significant to the public health profession and to the public every year. The meeting in Pittsburgh in 1939 will be especially noteworthy because the National Health Program will be launched in all probability during the coming year. This will be significant not only because of the funds available for expansion in public health but because of the likelihood that health departments generally will be the agencies to handle the new responsibilities for public medical care.

"The organized public health profession will have a large share of responsibility in carrying out the recommendations of the Technical Committee on Medical Care, and consequently in guiding and administering the expenditure of funds.

"The Association's meeting in Pittsburgh will provide the first opportunity for group expression of experiences, opinions and problems under which will be, in effect, a new public health regime.

We anticipate an attendance that will surpass all previous records."

Dr. Atwater announced that the Chairman of the Local Committee will be Dr. I. Hope Alexander, Director of Health of Pittsburgh.

The two items which follow were mailed Dec. 31, 1938, at Istanbul, and are printed by request of The Balkan Medical Union.

THE BALKAN MEDICAL UNION, in session at İstanbul, for the 5th Medical Week,

HAVING TAKEN INTO CONSIDERATION the terrible sufferings which a total war will bring upon the civil population of open towns together with the total lack of any adequate means of protection,

AND HAVING DISCOVERED that even in its restricted form the project of "sanitary towns" has not yet been adopted, and that all efforts made to protect civilians against chemical warfare have till now remained as proposals only, and that even the protocol prohibiting the use of asphyxiating gas has not yet been ratified by all nations,

HAS THEREFORE DECIDED to address itself to doctors of every nation with an appeal to take active measures and to fulfill this professional and humanitarian duty of awakening and stirring public opinion.

THE BALKAN MEDICAL UNION BELIEVES that only enlightened international opinion can make plain the imminence of the danger and the proved uselessness, even for the victor, of these terrible atrocities, and can thus lead to effective action.

The immutable truth that

HATE BREEDS ONLY HATE, AND ATROCITY BREEDS VENGEANCE must be impressed on everyone.

Prof. Dr. Bensis, Dr. Scaramanga (Athènes), Dr. Zika Markoviç, Prof. Dr. K. Sahoviç, Dr. M. Simoviç (Beograd), Prof. Dr. Gheorghiu, Dr. Popescu Buzeu (Bucarest), Prof. Dr. Akil Muhtar Özden, Prof. Sedat Tavat, Prof. Dr. A. Süheyl Ünver (İstanbul)

A Word About the Balkan Medical Union

The Balkan Medical Union is essentially a scientific organization. But it also represents an ideal. Its aim is to bring together the intellectuals of different countries so that they may know each other and come to a mutual respect and understanding, and in this way

form a compact block capable of opposing the misunderstanding that engender hate and disastrous struggles between nations.

This "Union" was formed in 1931 by a handful of men sincerely convinced that this humanitarian aim could and should be easily attained. Their conviction is based on the belief that this object is in the line of human evolution and that in helping this evolution the forward march can be hastened.

For intellectuals who have known how to see and grasp that which is the essence of humanity, what more beautiful ideal can be thought of than to work with all their faculties, all their energy to establish an understanding between men.

It is through the "Medical Weeks" that our Balkan Union tends to realize these aspirations.

Athens, Belgrad, Bucarest, and Istanbul, each in turn have seen more and more numerous and enthusiastic groups come together, all permeated by the same desire of comprehension and friendship. We doctors know that a very small quantity of vaccin can protect millions of men from the danger of contagious disease. So the good seed scattered by the Balkan Medical Union is a real remedy against the evils set loose by human passion.

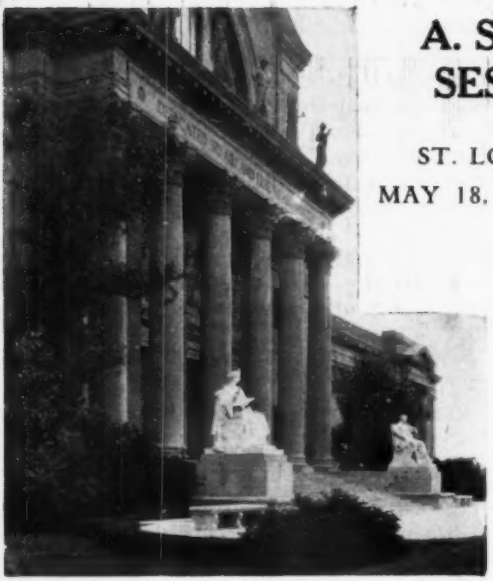
Prof. Dr. Âkil Moukhtar Özden

NATIONAL

New Hampshire

On May 17, 1938, a group of New Hampshire medical technologists met in Manchester for the purpose of organization. The society was named, its purpose stated and officers for the year were elected. Dr. Frank Connell, Professor of Parasitology, Dartmouth Medical School, gave a lecture and demonstrated technic in the study of intestinal parasites in man. The list of officers elected is as follows:

Mrs. Evelyn Jardine, M.T., Hanover, N. H., President; Miss Lily Ford, M.T., Dover, N. H., Treasurer; Miss Harriet M. Boyd, M.T., Wolfeboro, N. H., Secretary.



Art Museum, St. Louis, Mo.

A. S. M. T. SESSION

ST. LOUIS, MO.

MAY 18, 19, 20, 1939

HOTEL RESERVATIONS

For your convenience in making hotel reservations for the St. Louis session of the American Society of Medical Technologists, use reservation form below:

TO: WM. H. SCHNEIDER, Convention Manager
Hotel Jefferson, Twelfth & Locust Sts., St. Louis, Mo.

PLEASE RESERVE FOR ME ACCOMMODATIONS AS CHECKED

- ☐ **Two Connecting Rooms with Bath Between for Two....$2.50 per Room**
- ☐ **Single Room with Bath.....$3.00, $3.50, $4.00, $4.50, $5.00, $5.50**
- ☐ **Double Rooms with Bath, Double Bed.....$4.00, $4.50, $5.00, $5.50, $5.00, $7.00, $7.50**
- ☐ **Double Rooms with Bath, Twin Beds....$5.00, $5.00, $7.00, $7.50, $8.00**
- ☐ **Suites.....$6.00, $8.00, $10.00**

Check Style of Room Desired and Underscore Rate Rate $.....

Immediate reservation necessary to assure accommodations at your headquarters hotel

Be sure to give arrival and departure date.

Will arrive on (Date).....Will depart on (Date).....

Name.....

Street.....

City and State.....

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS CONVENTION

MAY 18, 19, 20, 1939